

15.

**CLAIMS**

1. A device comprising a well defined within a substrate, said substrate comprising, in sequence, a first base layer, a second hydrophobic layer, and a third hydrophilic layer; said well extending from the upper surface of the base layer through the second and third layers to provide an opening in the upper surface of the third layer wherein a lipid membrane comprising a closely packed array of self-assembling amphiphilic molecules extends across the well within the region defined between the first base layer and the third hydrophilic layer.  
5
2. A device according to claim 1, wherein the lipid membrane is composed such that the impedance of the membrane is dependent on the presence or absence of an analyte to be detected.  
10
3. A device according to claim 1 or claim 2, wherein the dimensions of the well are selected such that a bead of polar liquid retained by the well is of sufficient size to prevent contact of the membrane with air, but still be capable of rapid exchange with analyte solutions or other solutions.  
15
4. A device according to any one of claims 1 to 3 wherein the opening is substantially circular and has a radius of from about 10 to 200 microns.
5. A device according to any one of claims 1 to 4, wherein the first base layer is from 50 nm to 150 nm thick.  
20
6. A device according to any one of claims 1 to 5, wherein the second hydrophobic layer is from 100 nm to 300 nm thick.  
25
7. A device according to any one of claims 1 to 6, wherein the third hydrophilic layer is from 400 nm to 600 nm thick.
8. A device according to any one of claims 1 to 7, wherein the first base layer is a conductive layer.
9. A device according to claim 8, wherein the conductive layer is formed from gold.

## 16.

10. A device according to any one of claims 1 to 9, wherein the device acts as a component in an electrode sensor.
11. A device according to any one of claims 1 to 10, wherein the third hydrophilic layer comprises a hydrophilic material selected from the group consisting of silicon carbide, silicon oxide, silicon dioxide, titanium dibromide, titanium oxide, titanium nitride, zinc oxide, zirconium dioxide, magnesium oxide, iron oxides, graphite, boron nitride, chromium nitride, and poly vinylidene fluoride.  
5
12. A device according to claim 11, wherein the hydrophilic material is titanium oxide.
13. A device according to any one of claims 1 to 12, wherein the second hydrophobic layer comprises a hydrophobic material selected from the group consisting of polyamides, PVC, polystyrenes, polyesters, polycarbonates, polyurethanes, nylons Glass fibre, Plastics, Silicon rubbers, Latex, glass, vinyl, phenolic, resins, brass, Tetrafluoroethylcne Octadecyltrichlorosilane, Teflon, Silicon nitride, Silicon carbide, aluminium nitride, oxidised silicon carbide, Butadiene Styrene, Ethylene vinyl acetate, and PTFE (polytetrafluoroethylcne) polymer.  
10
14. A device according to claim 13, wherein the hydrophobic material is oxidised silicon carbide.
15. A device according to any one of claims 1 to 14, wherein the third hydrophilic layer comprises titanium oxide and the second hydrophobic layer comprises oxidised silicon carbide.  
20
16. A device according to any one of claim 1 to 15, wherein a mesh covers at least a portion of the well opening.
17. A device according to claim 16, wherein the mesh forms a tesselating pattern.
18. A device according to claim 17, wherein the tesselating pattern is hexagonal.
- 25 19. A device according to any one of claims 16 to 18, wherein the wall partitions of the mesh are from 1nm to 10 nm in thickness and the individual cells are from 20 nm to 100 nm wide at their widest point.

17.

20. A device according to any one of claims 1 to 19, comprising a plurality of wells in a single substrate.
21. A device according to claim 21 comprising sixteen wells in a single substrate.
22. A device according to any one of claims 1 to 21 wherein the device further comprises  
5 a support layer.
23. A device according to claim 22 wherein the support layer comprises a silicon support and a titanium layer.
24. A device according to any one of claims 1 to 23 wherein the lipid membrane  
comprises a lower first membrane layer and an upper second membrane layer and  
10 wherein the lipid membrane further comprises a plurality of ionophores with at least  
a proportion of the molecules and ionophores of the lower first layer being connected  
to the upper surface of the first base layer by means of linker groups.
25. A device according to any one of claims 1 to 24 wherein the device further comprises  
15 a fourth hydrophobic layer and wherein said well further extends to the upper  
surface of the fourth layer.
26. A device according to claim 25, wherein the fourth hydrophobic layer is from 100 nm  
to 300 nm thick.
27. A device according to any one of claims 25 to 26, wherein the fourth hydrophobic  
layer comprises a hydrophobic material selected from the group consisting of  
20 polyamides, PVC, polystyrenes, polyesters, polycarbonates, polyurethanes, nylons,  
Glass fibre, Plastics, Silicon rubbers, Latex, glass, vinyl, phenolic, resins, brass,  
Tetrafluoroethylone Octadecyltrichlorosilane, Teflon, Silicon nitride, Silicon carbide,  
aluminium nitride, oxidised silicon carbide, Butadiene Styrene, Ethylene vinyl  
acetate, and PTFE (polytetra fluoroethylene) polymer.
- 25 28. A device according to claim 27, wherein the hydrophobic material is oxidised silicon  
carbide.

18.

29. A device according to any one of claims 25 to 28, wherein the internal circumference of the well is varied such that it is greater in the region defined by the fourth hydrophobic layer than in the region defined by the second hydrophobic layer.
30. A membrane-based biosensor comprising a device according to any one of claims 1 to 29.
31. A method of forming a device comprising the steps of:
  - (i) depositing a first base layer;
  - (ii) depositing a second hydrophobic layer on the first base layer;
  - (iii) depositing a third hydrophilic layer on the second hydrophobic layer to form a substrate;
  - (iv) forming a well in the substrate extending from the upper surface of the base first layer through the second and third layers to provide an opening in the upper surface of the third layer; and
  - (v) forming a lipid membrane comprising a closely packed array of self-assembled amphiphilic molecules within the well such that it extends across the well within the region defined between the first base layer and the third hydrophilic layer.
32. A method according to claim 31 wherein the first base layer is deposited on the layer of titanium of a support material comprising a silicon support and the layer of titanium.
33. A method according to claim 32 wherein the support material is formed by depositing a layer of titanium on the silicon support.
34. A method according to claim 32 or claim 33 wherein the silicon support is a single crystal silicon wafer.
35. A method according to any one of claims 31 to 34, wherein the first base layer is from 50 nm to 150 nm thick.

19.

36. A method according to any one of claims 31 to 35, wherein the second hydrophobic layer is from 100 nm to 300 nm thick.
37. A method according to any one of claims 31 to 36, wherein the third hydrophilic layer is from 400 nm to 600 nm thick.
- 5 38. A device according to any one of claims 31 to 37, wherein the first base layer is a conductive layer.
39. A device according to claim 38, wherein the conductive layer is formed from gold.
40. A device according to any one of claims 31 to 39, wherein the third hydrophilic layer comprises a hydrophilic material selected from the group consisting of silicon carbide, silicon oxide, silicon dioxide, titanium dibromide, titanium oxide, titanium nitride, zinc oxide, zirconium dioxide, magnesium oxide, iron oxides, graphite, boron nitride, chromium nitride, and poly vinylidene fluoride.
- 10 41. A device according to claim 40, wherein the hydrophilic material is titanium oxide.
42. A device according to any one of claims 31 to 41, wherein the second hydrophobic layer comprises a hydrophobic material selected from the group consisting of polyamides, PVC, polystyrenes, polyesters, polycarbonates, polyurethanes, nylons, Glass fibre, Plastics, Silicon rubbers, Latex, glass, vinyl, phenolic, resins, brass, Tetrafluoroethylene Octadecyltrichlorosilane, Teflon, Silicon nitride, Silicon carbide, aluminium nitride, oxidised silicon carbide, Butadiene Styrene, Ethylene vinyl acetate, and PTFE (polytetrafluoroethylene) polymer.
- 15 43. A device according to claim 42, wherein the hydrophobic material is oxidised silicon carbide.
44. A method according to any one of claims 31 to 43 wherein the well is formed by etching.
- 20 45. A device according to any one of claims 31 to 44 wherein the method further comprises the step of depositing a fourth hydrophobic layer on the third hydrophilic

20.

layer wherein said well is formed so as to extend to the upper surface of the fourth layer.

46. A method according to claim 45, wherein the fourth hydrophobic layer is from 100 nm to 300 nm thick.
- 5 47. A device according to any one of claims 45 to 46, wherein the fourth hydrophobic layer comprises a hydrophobic material selected from the group consisting of polyamides, PVC, polystyrenes, polyesters, polycarbonates, polyurethanes, nylons, Glass fibre, Plastics, Silicon rubbers, Latex, glass, vinyl, phenolic, resins, brass, Tetrafluoroethylene Octadecyltrichlorosilane, Teflon, Silicon nitride, Silicon carbide, 10 aluminium nitride, oxidised silicon carbide, Butadiene Styrene, Ethylene vinyl acetate, and PTFE (polytetrafluoroethylene) polymer.
48. A device according to claim 47, wherein the hydrophobic material is oxidised silicon carbide.
49. A method according to any one of claims 31 to 48 wherein the lipid membrane comprises a lower first membrane layer and an upper second membrane layer and wherein the lipid membrane further comprises a plurality of ionophores with at least a proportion of the molecules and ionophores of the lower first layer being connected to the upper surface of the first base layer by means of linker groups.
50. A method according to claim 49 wherein the step of forming the lipid membrane comprises: forming a first solution containing one or more amphiphilic molecules, one or more linker groups and one or more ionophores in a first organic solvent; contacting the first base layer of the well with the first solution to form the lower first membrane layer comprising a closely packed array of said amphiphilic molecules and said ionophores wherein said lower first membrane layer is connected to the first base layer by means of said linker groups; rinsing the device with a suitable second organic solvent; removing the excess second organic solvent; forming a second solution of one or more amphiphilic molecules and one or more ionophores in a suitable third organic solvent; contacting the second solution with the device comprising said first lower membrane layer to form the second layer membrane

## 21.

layer; rinsing the device with an aqueous solution; and removing the device from the aqueous solution and allowing to drain.

51. A method according to claim 50, wherein said first organic solvent, said second organic solvent and said third organic solvent are each ethanol.
- 5 52. A method according to claim 50 or claim 51 wherein said second organic solvent is removed by rapid air drying.
53. A method according to claim anyone of claims 50 to 52, wherein immediately upon removal of the excess second organic solvent, the device is immersed in the third solution.
- 10 54. A method according to any one of claims 50 to 53 wherein the one or more ionophores comprise gramicidin A or an analogue thereof.
55. A method according to claim 54, wherein the one or more ionophores are biotinylated.
- 15 56. A method according to any one of claims 50 to 55, wherein one or more receptors are attached to the surface of the membrane.
57. A method according to claim 56, wherein the one or more receptors are attached to the membrane by using streptavidin, avidin or one of the related biotin binding-proteins.
- 20 58. A method according to claim 56 or claim 57, wherein the one or more receptors are coupled to one or more biotinylated gramicidin ion channels and/or one or more biotinylated membrane-spanning lipid.
59. A method of preparing a membrane-based biosensor comprising the steps of:
  - (a) adding a solution of streptavidin, avidin, neutravidin, avidin or streptavidin derivative onto the surface of a device according to any one of claims 1 to 28 wherein the lipid membrane of said device comprises one or more biotinylated gramicidin ion channels and/or one or more biotinylated membrane spanning lipids;

22.

- (b) rinsing the device with an aqueous solution in order to remove excess streptavidin, avidin, neutravidin or other avidin or streptavidin derivative;
  - (c) adding a solution of a biotinylated receptor molecule so that the receptor molecules attach to the membrane via the biotin-streptavidin-biotin link;
  - 5 (d) rinsing the membrane with an aqueous solution;
  - (e) removing the device from the aqueous solution and allowing to drain, such that a bead of aqueous solution is retained within the well of the device.
60. A method according to claim 59, wherein following step (e) the electrode is stored at between minus 20°C and plus 5°C.